Journal of Medical Virology 58:359–365 (1999)

Expression of Interferon Receptor Genes in the Liver as a Predictor of Interferon Response in Patients With Chronic Hepatitis C

Katsumi Morita, Katsuaki Tanaka,* Satoru Saito, Takehiko Kitamura, Takayoshi Kiba, Takando Fujii, Kazushi Numata, and Hisahiko Sekihara

Third Department of Internal Medicine, Yokohama City University School of Medicine, Yokohama, Japan

Interferon (IFN) receptor mRNA expression patterns in the liver have been shown to correlate with the effectiveness of IFN therapy in patients with hepatitis C virus (HCV) infection. However, it is not clear to what extent this factor contributes to the short (primary)- and long (sustained)-term results of IFN treatment with respect to biochemical and virological remission. Eighty-two patients who subsequently received lymphoblastoid IFN-α therapy underwent liver biopsies before IFN therapy. Possible factors that might correlate with IFN response were chosen and analyzed. The primary biochemical and virological responses at the end to treatment (24 weeks) were 63% and 43% vs. 46% and 32% for sustained biochemical and virological remission at the end of follow-up (48 weeks), respectively. In univariate analysis, the absence of HCV genotype 1b, a low titer of HCV RNA, and the expression of IFN receptor mRNA were significantly correlated with sustained biochemical and virological responses to IFN therapy. Multiple logistic regression analysis showed that IFN receptor mRNA expression and the absence of genotype 1b were significant predictors of the sustained biochemical and virological effectiveness of IFN therapy. IFN receptor mRNA expression predicted a sustained virological response to IFN therapy with a positive predictive value of 100% with genotype non-1b and had a negative predictive value of 97% with genotype 1b. It is concluded that expression of IFN receptor genes in the liver is a useful index for predicting the short- and long-term efficacy of IFN therapy in patients with chronic HCV infection. ***J. Med. Virol. 58:359–365, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: IFNAR1; IFNAR2; alanine transaminase; genotype; polymerase; chain reaction; mRNA

INTRODUCTION

Hepatitis C virus (HCV) is the major etiologic agent of human viral hepatitis [Kuo et al., 1989] and is associated with the development of cirrhosis and hepatocellular carcinoma [Dienstag, 1983; Kiyosawa et al., 1990]. Interferon (IFN) has been used for the treatment of chronic hepatitis C [Hoofnagle et al., 1986; Davis et al., 1989; Di Bisceglie et al., 1989]. Approximately 50% of patients respond to therapy, however, and in half of those the serum aminotransferase concentration returns to normal with IFN treatment relapse after cessation of therapy [Tine' et al., 1991; Marcellin et al., 1991]. Several factors have been reported to be predictive of response to IFN therapy in chronic hepatitis C [Davis, 1994]: a low titer of HCV, the presence of HCV genotype 2a or 2b (HCV-2a or HCV-2b), less severe hepatitis, and mutations in nonstructural protein 5A (between codons 2209 and 2248) ($NS5A_{2209\text{-}2248}$) sequences in HCV genotype 1b (HCV-1b) infection [Enomoto et al., 1996; Chayama et al., 1997].

The IFN molecule itself has no antiviral effects and the effects of IFN are mediated through interaction with a specific multisubunit cell surface receptor [Aguet, 1980; Colamonici et al., 1992]. Some researchers have suggested that expression of IFN receptor genes in the liver correlates with the effectiveness of IFN therapy in patients with HCV infection [Fukuda et al., 1997; Yatsuhashi et al., 1997]. Recently, we reported that, in patients with HCV-2a or HCV-2b infection, IFN receptor gene expression in the liver was associated significantly with the efficacy of IFN using multiple logistic regression analysis [Morita et al., 1998]. However, among chronic hepatitis C patients including HCV-1b infection, it is not clear to what extent these predictive factors contribute to a favorable

*Correspondence to: Katsuaki Tanaka, MD, Third Department of Internal Medicine, Yokohama City University School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236, Japan.

Accepted 11 January 1999

response to IFN therapy or how they relate to each other. In this study, various factors that may correlate with IFN response, including HCV genotype, pretreatment virus load, $NS5A_{2209\text{-}2248}$ sequences, histological features, and hepatic IFN receptor mRNA expression, were analyzed in patients with HCV infection, and the short (primary)- and long (sustained)-term results of IFN treatment, as well as the effect of such relevant factors on response to IFN therapy, are described.

MATERIALS AND METHODS

Patient Population

Eighty-two patients with chronic HCV infection were treated at Yokohama City University Hospital between July 1994 and September 1997. There were 51 men and 31 women, aged from 21 to 68 years (mean: 47 years). All patients had had abnormal alanine transaminase (ALT) levels for more than 6 months, and chronic hepatitis was confirmed by liver biopsy. No patients had a history of alcohol or drug abuse or evidence of metabolic or autoimmune disorders, and none had received previously corticosteroid, immunosuppressive, or antiviral treatment. All patients received a 24-week course of IFN-α. Six million units of natural IFN-α (human lymphoblastoid interferonl Sumitomo Pharmaceutical Co., Osaka, Japan) were administered intramuscularly to 75 patients and 5 million units of natural IFN-α (human lymphoblastoid interferon; Otsuka Pharmaceuticals Co., Tokyo, Japan) were administered to 7 patients for an initial 14-day period, and then 3 times a week for 22 weeks. During the course of IFN therapy, there were no severe side effects in any patients and no patients had their IFN doses reduced. In all patients, liver biopsy was performed before IFN treatment. Liver samples were immediately frozen in liquid nitrogen and stored at −80°C until analysis. Using pretreatment serum samples, HCV genotypes and HCV concentrations were determined as described previously [Morita et al., 1998]. Biochemical and virological responses to IFN treatment were assessed at the end of the treatment period (primary response), at week 24, and at the end of the follow-up period (sustained response), week 48. A biochemical response was defined as serum ALT concentrations within the normal range, and a virological response as the absence of serum HCV RNA by reverse transcription-polymerase chain reaction (RT-PCR) method. The study was carried out according to the guidelines of the Helsinki Declaration. Informed consent was obtained from all patients and their relatives.

Determination of Hepatic IFN Receptor Gene Expression

Using pretreatment liver samples, 2 subunits of the type I IFN receptor, IFNAR1 (IFN-α receptor), and IFNAR2 (IFN-α/β receptor) [Uzé et al., 1990; Novick et al., 1994; Lutfalla et al., 1995] were determined by RT-nested PCR as reported previously [Morita et al., 1998]. The sequences of primers for IFNAR1 were: outer sense, 5′-AGTGTTATGTGGGCTTTGGATGGTTTAAGC- 3′ (nucleotides [nt] 535–564); outer antisense, 5′-TCTGGCTTTCACACAATATACAGTCAGTGG-3′ (nt 1270–1299); inner sense, 5′-GACCTTTCAAGTTCAGTGGCTCCACGCC-3′ (nt 843–870); and inner antisense, 5′-GGATCACAGGCGTGTTTCCAGACTG-3′ (nt 1141–1165). The sequences of primers used for IFNAR2 mRNA were: outer sense, 5′-GCTTTTGAGCCAGAATGCCT-3′ (nt 329–348); outer antisense, 5′-CCCTCTGACTGTTCTTCAATG-3′ (nt 833–853); inner sense, 5′-GAAGGTGGTTAAGAACTGTGC-3′ (nt 563–583); and inner antisense, 5′-CCCGCTGAATCCTTCTAGGACGG-3′ (nt 649–671). PCR amplification was performed by 35 cycles of denaturation for 1 min at 94°C, annealing for 45 sec at 55°C, and extension for 2 min at 72°C. Glycerol-3-phosphate dehydrogenase (G3PDH) mRNA was used to confirm successful RNA extraction from liver specimens and as a positive control for the RT-PCR assay. The final PCR products were subjected to electrophoresis on a 2% agarose gel and the bands were visualized using ethidium bromide staining.

Determination of Nucleotide Sequence of the $NS5A_{2209\text{-}2248}$ Gene

Using pretreatment serum samples from patients with HCV-1b infection, serum RNA was extracted for the determination of the nucleotide sequence of the $NS5A_{2209\text{-}2248}$ gene. Nucleotides 6703–7320 (numbered on the basis of the sequence of HCV-J [Kato et al., 1990]) of HCV RNA were amplified by RT-nested PCR. To determine the nucleotide sequence of this region, direct sequencing was performed on an automated DNA sequencer (377 DNA Sequencer, Perkin-Elmer Cetus, Norwalk, CT). The resulting amino acid sequences of $NS5A_{2209\text{-}2248}$ were compared with the $NS5A_{2209\text{-}2248}$ sequence identified in HCV-J. The procedures were carried out after the method reported by Enomoto et al. [1996].

Histologic Examination

Histologic classification was according to the European classification of chronic hepatitis [Groote et al., 1968]. To assess histologic activity, the histologic activity index (HAI) score was used [Knodell et al., 1981].

Statistical Analysis

Data are expressed as means ± SE. Conventional statistical analysis utilized the Student's *t*-test or chi-squared test. Changes in serum ALT levels as measured monthly were assessed by a 2-way repeated-measures analysis of variance. Multivariate assessment by logistic regression was carried out to determine independent characteristics associated with the efficacy of IFN therapy. A multiple logistic regression model was built using the following explanatory variables: age, sex (female = 0, male = 1), serum ALT concentration, HCV genotype (genotype 1b = 0, genotype non-1b = 1), serum HCV RNA concentration, conventional histologic diagnosis (chronic active hepatitis [CAH] 2A = 0, CAH 2B = 1), HAI score, and IFN

TABLE I. Clinical Features of Patients Before IFN Treatment

Parameter	
No. of patients	82
Age (years)[a]	47 ± 1.4
Sex (female/male)	31/51
ALT (IU/l)[a]	100 ± 8.5
Genotype	
1b	50
Non-1b (2a or 2b)	32
HCV RNA concentration[a,b]	5.6 ± 0.1
Histology	
CAH 2A	58
CAH 2B	24
HAI score[a]	4.3 ± 0.3

[a]Data are means ± SE.
[b]Data are expressed as $\log_{10}$ (number of copies/ml serum).

receptor mRNA expression in the liver (negative = 0, positive = 1). Data analysis was carried out with the computer program SPSS (SPSS Japan, Tokyo, Japan). All P values were 2-sided and values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Clinical Features of Patients Before IFN Treatment

The characteristics of participating patients (baseline clinical and laboratory data) are summarized in Table I. The prevalence of HCV-1b was 61% (50/82 patients) and that of HCV-non-1b (HCV-2a or 2b) was 39% (32/82 patients), and the pretreatment levels of serum HCV RNA and serum ALT levels were 5.6 ± 0.1 $\log_{10}$ copies/ml and 100 ± 8.5 IU/l, respectively.

IFNAR1 and IFNAR2 mRNA Expression in the Liver

In the liver samples containing IFNAR1 and IFNAR2 mRNA, PCR generated a single band of DNA of the expected size (323 and 109 bp, respectively). The prevalence of IFNAR1 mRNA was 35% (29/82 patients) and that of IFNAR2 mRNA was 40% (33/82 patients). Both IFNAR1 and IFNAR2 mRNA were detectable in the liver of 25 patients, IFNAR1 mRNA alone was detectable in 4 patients, IFNAR2 mRNA alone was detectable in 8 patients, and both IFNAR1 and IFNAR2 mRNA were absent in 45 patients. The expression rates of both IFNAR1 and IFNAR2 mRNA, which we defined as "IFN receptor mRNA-positive," were 30% (25/82 patients) and those of the absence of either IFNAR1 or IFNAR2 mRNA or both, which we defined as "IFN receptor mRNA-negative," were 70% (57/82 patients).

Primary (End of Treatment) and Sustained (End of Follow-Up) Responses to IFN Treatment and Hepatic IFN Receptor mRNA Expression

At the end of IFN therapy, 52 patients (63%) were in a primary biochemical remission and 38 patients (46%) were in a primary virological remission (Table II). All patients (100%) in the IFN receptor mRNA-positive group and 27 patients (47%) in the IFN receptor mRNA-negative group were in biochemical remission and the difference was highly significant ($P < 0.0001$). At the same time, virological response was observed in all patients (100%) in the IFN receptor mRNA-positive group and in 13 patients (23%) in the IFN receptor mRNA-negative group, the difference also being highly significant ($P < 0.0001$). At the end of follow-up, 38 patients (46%) were in a biochemical remission and 26 patients (32%) were in a virological remission. Twenty-one patients (84%) in the IFN receptor mRNA-positive group remained in biochemical remission and 19 (76%) remained in virological remission. Conversely, only 17 patients (30%) in the IFN receptor mRNA-negative group had normal serum ALT levels, and only 7 (12%) were serum HCV-RNA-negative. The proportion of patients with biochemical and virological responses at the end of the follow-up period differed significantly between the IFN receptor mRNA-positive and -negative groups ($P < 0.0001$ for both).

IFN receptor mRNA-positive patients tended to have higher rates of HCV-non-1b infection (14/25 patients; 56%) than IFN receptor mRNA-negative subjects (18/57 patients; 32%), however, there were no significant differences between the 2 groups. With regard to serum pretreatment HCV RNA concentrations, there were no significant differences between patients positive for IFN receptor mRNA (5.3 ± 0.2 $\log_{10}$ copies/ml) and those negative (5.7 ± 0.1 $\log_{10}$ copies/ml). There were no significant HAI score differences between patients positive for IFN receptor mRNA (4.4 ± 0.5) and those negative (4.3 ± 0.3). Multiple logistic regression analysis revealed that no factors significantly influenced IFN receptor mRNA expression (data not shown). Figure 1 shows the serial changes in serum ALT levels in patients positive and negative for IFN receptor mRNA. Serum ALT levels decreased significantly from their basal levels in patients positive for IFN receptor mRNA compared with negative patients ($P = 0.0036$).

Factors Predicting Response to IFN Therapy

Table III shows factors associated with a sustained biochemical or virological response to IFN by univariate analysis. There was a significant positive association for both responses with IFN receptor mRNA positivity and the absence of HCV-1b, and a negative association with baseline serum HCV RNA levels. Multiple logistic regression analysis was used to assess a variety of variables that might contribute to sustained biochemical and virological responses to IFN (Table IV). The model showed positivity for IFN receptor mRNA and the absence of HCV-1b to be independent predictors of the effectiveness of IFN therapy, and that IFN receptor mRNA expression most efficiently contributed to the treatment outcome in both response groups. Other clinical factors, such as age, sex, HCV RNA concentration, serum ALT levels, histology, and HAI score were not independent factors. Although pretreatment serum HCV RNA concentration was correlated with the sustained response to IFN in univariate

TABLE II. Primary (End of Treatment) and Sustained (End of Follow-Up) Responses to IFN Treatment and Hepatic IFN Receptor mRNA Expression

	IFN receptor mRNA (%)			
	Positive	Negative	Total (%)	P^a
Primary response				
Biochemical	25/25 (100)	27/57 (47)	52/82 (63)	<0.0001
Virological	25/25 (100)	13/57 (23)	38/82 (46)	<0.0001
Sustained response				
Biochemical	21/25 (84)	17/57 (30)	38/82 (46)	<0.0001
Virological	19/25 (76)	7/57 (12)	26/82 (32)	<0.0001

[a]Chi-squared test.

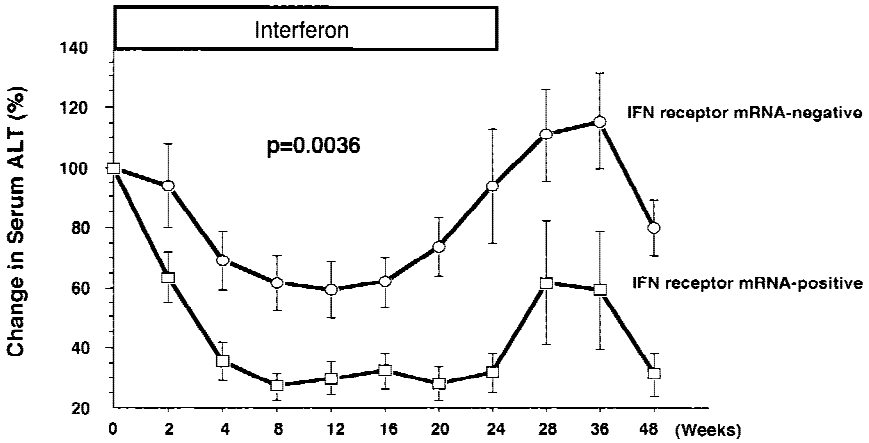

Fig. 1. Changes in serum ALT levels in patients positive or negative for IFN receptor mRNA. Changes are expressed as means ± SE from pretreatment values. Serum ALT levels decreased significantly more from basal levels in patients positive for IFN receptor mRNA than those negative. The differences in average percent change between the 2 groups were significant (39 vs. 82%, $P = 0.0036$).

TABLE III. Factors Associated With a Sustained Biochemical or Virological Response to IFN by Univariate Analysis

Variable (association)	Sustained response (P)	
	Biochemical	Virological
Age	NS[a]	NS
Sex	NS	NS
Baseline ALT level	NS	NS
Absence of HCV-1b	0.0106	<0.0001
Baseline HCV RNA level (low)	0.0044	0.0007
Histology	NS	NS
HAI score	NS	NS
IFN receptor mRNA (positive)	<0.0001	<0.0001

[a]NS, not significant.

analyses, in multivariate analyses it was not an independent predictor.

Mutations in the NS5A Gene and Response to IFN in Patients With HCV-1b Infection

The amino acid sequences of $NS5A_{2209\text{-}2248}$ in patients with HCV-1b infection and the expression of IFN receptor mRNA are shown in Figure 2. The sequences of only 47 patients are shown because cDNA from serum samples of 3 patients could not be amplified using our primers. All patients were divided into 3 categories by the number of mutations in the NS5A gene, as described by Enomoto et al. [1996]. Twenty-six (55%) patients had the wild-type sequence, with no amino acid changes; 19 (40%) patients had the intermediate type, with 1–3 amino acid changes; and 2 (4%) patients had the mutant type, with 4 or more changes. However, there were no significant differences between the sustained virological responders and nonresponders with respect to the number of amino acid substitutions in the $NS5A_{2209\text{-}2248}$ region. With respect to the correlation between the number of amino acid substitutions in the $NS5A_{2209\text{-}2248}$ region and IFN receptor mRNA expression, there were no significant differences between patients positive for IFN receptor mRNA and those negative, and the number of amino acid changes in the $NS5A_{2209\text{-}2248}$ region did not correlate with serum HCV RNA levels (data not shown).

Relationship Between HCV Genotype and IFN Receptor mRNA Expression Contributing to IFN Response

The relationship between 2 predictive factors (HCV genotype and hepatic IFN receptor mRNA expression) contributing to primary and sustained virological responses is shown in Table V. The presence of IFN re-

TABLE IV. Analysis of Factors That Contribute to the Efficacy of IFN Therapy Using Multiple Logistic Regression Analysis

	Sustained biochemical response/sustained virological response			
Explanatory variable	Estimate value[a]	SE	*P*	R statistic[b]
IFN receptor mRNA-positive	2.7225/5.3404	0.7561/1.4430	0.0003/0.0002	0.3112/0.3379
Absence of HCV-1b	1.9731/3.6546	0.7454/3.6546	0.0081/0.0031	0.2103/0.2569
Age	–0.0476/–0.0750	0.0255/0.0402	0.0618/0.0594	–0.1146/–0.1232
HCV RNA concentration	–0.3332/–0.9823	0.3853/0.5854	0.3872/0.0934	0.0000/–0.0892

[a]Partial regression coefficiency.
[b]Represents the degree of the partial contribution of each variable to the model. R statistics can range in value from –1 to 1; –1 or +1 means maximum contribution.

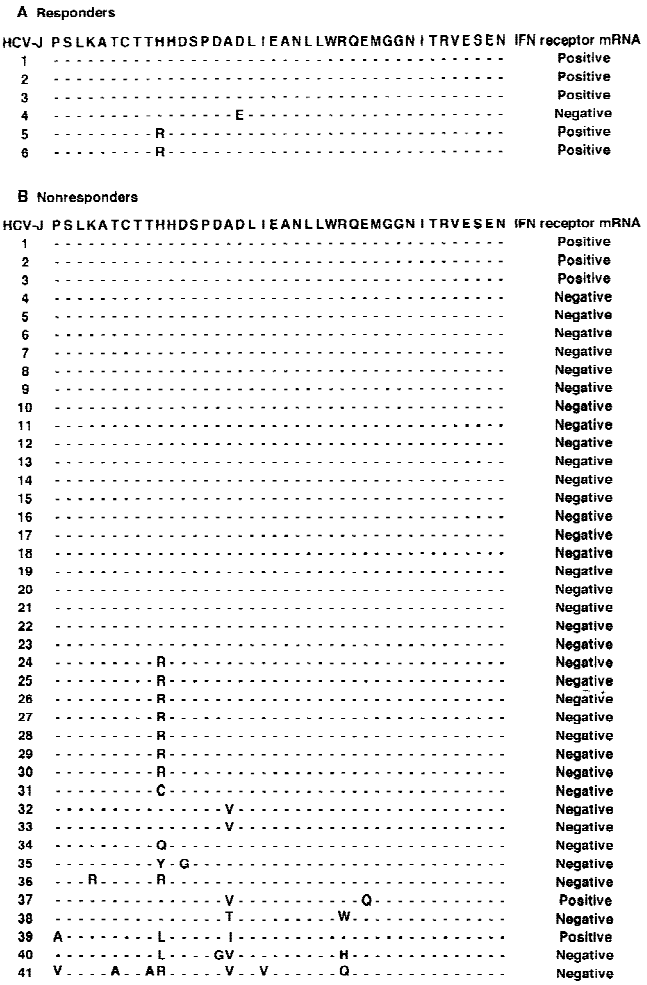

Fig. 2. The amino acid sequences of $NS5A_{2209\text{-}2248}$ in sustained virological responders **(A)** and nonresponders **(B)** and the expression of IFN receptor mRNA. The sequences are compared with those of prototype HCV genotype 1b (HCV-J) reported by Kato et al. [1990]. There were no significant differences between patients positive for IFN receptor mRNA and those negative with respect to the number of amino acid substitutions in the $NS5A_{2209\text{-}2248}$ region.

ceptor mRNA predicted a primary virological response to IFN treatment with a positive predictive value of 100% in both patients with HCV-1b infection and those with HCV-non-1b infection. In contrast, IFN receptor mRNA expression predicted a sustained virological response to IFN therapy with a positive predictive value of 100% with genotype non-1b and had a negative predictive value of 97% with genotype 1b.

DISCUSSION

Since IFN therapy is expensive and may cause serious adverse effects, it would be clinically useful to be able to predict the efficacy of IFN in patients with HCV infection [Davis, 1994]. Using multivariate analysis, serum virus levels [Hagiwara et al., 1993] or HCV genotype, serum virus levels, and hepatitis activity [Suzuki et al., 1995] have been found to be predictive independently of the response to IFN therapy. In the present study, we demonstrated that IFN receptor mRNA expression in the liver and HCV genotype (absence of HCV-1b) were independent predictors of short- and long-term efficacy of IFN therapy in patients with chronic HCV infection, and that IFN receptor mRNA expression most efficiently contributed to the treatment outcome.

The results also show that IFN receptor mRNA expression predicted the primary virological response to IFN in the same manner regardless of HCV genotypes; however, it predicted the sustained virological response to IFN differently between the patients with HCV-1b infection and those with HCV-non-1b infection. In patients with HCV-1b infection, IFN therapy could not eradicate HCV completely in 6 patients despite the presence of IFN receptor mRNA in the liver, while in patients with HCV-non-1b infection, a sustained favorable response to IFN therapy was obtained in all patients with expression of IFN receptor mRNA in the liver. The reason for this difference has not been clarified. Two possible explanations are 1) a difference in intracellular signal transduction after receptor binding and 2) the presence of an IFN sensitivity determining region (ISDR) [Enomoto et al., 1996; Chayama et al., 1997] in patients with HCV-1b infection. The former explanation depends on the fact that there is disruption of subsequent signal transduction after receptor binding, because some IFN-resistant cells may express functional receptors [Aguet et al., 1981]. Therefore, resistance to antiviral effects may not be regulated at the receptor level and expression of IFN receptor genes may be a necessary first step but not sufficient to obtain a physiological response, especially in patients with HCV-1b infection. The latter explanation may depend on the fact that the mutations in the

TABLE V. Relationship Between HCV Genotype and IFN Receptor mRNA Expression Contributing to IFN Response*

HCV genotype	IFN receptor mRNA	Response to IFN treatment	
		Primary virological response	Sustained virological response
1b	Positive	11 of 11 patients, PPV = 100%	5 of 11 patients, PPV = 45%
1b	Negative	6 of 39 patients, NPV = 85%	1 of 39 patients, NPV = 97%
Non-1b	Positive	14 of 14 patients, PPV = 100%	14 of 14 patients, PPV = 100%
Non-1b	Negative	7 of 18 patients, NPV = 61%	6 of 18 patients, NPV = 67%

*PPV, positive predictive value = (true positive/{true positive + false positive}); NPV, negative predictive value = (true negative/{true negative + false negative}).

$NS5A_{2209\text{-}2248}$ gene were related to a favorable response to IFN treatment in Japanese patients with HCV-1b infection [Enomoto et al., 1996; Chayama et al., 1997]. However, in the present study, the response to IFN therapy was not related to the number of amino acid substitutions in the $NS5A_{2209\text{-}2248}$ region in patients with HCV-1b infection. These data may be in contrast to the hypothesis that $NS5A_{2209\text{-}2248}$ represents an ISDR [Enomoto et al., 1996; Chayama et al., 1997], but are consistent with data from outside of Japan [Squadrito et al., 1997; Zeuzem et al., 1997], although further studies of this issue are required. In the present study, all 6 patients with HCV-1b infection, who expressed IFN receptor mRNA in the liver but showed neither a sustained biochemical nor a virological response to IFN therapy, responded partially to IFN therapy; i.e., they showed primary biochemical and virological responses and their serum ALT levels at the end of follow-up tended to be lower than those of IFN receptor-negative patients (data not shown). Therefore, expression of IFN receptor mRNA in the liver contributes to the antiviral effects on HCV replication and biochemical remission, but may not be sufficient for the complete clearance of HCV genome in some cases of HCV-1b infection.

It is also unclear why 6 responders with HCV-non-1b infection and 1 responder with HCV-1b infection did not express IFN receptor mRNA. A possible explanation is that IFNAR1 or IFNAR2 mRNA levels in the liver were below the sensitivity of our RT-PCR assay, rather than being absent in those liver samples, because peripheral blood mononuclear cells expressed both IFNAR1 and IFNAR2 mRNA in the same patients (data not shown).

In univariate analysis, a low titer of HCV RNA, the absence of HCV-1b, and IFN receptor mRNA expression were significantly correlated with a sustained response to IFN therapy; however, a low titer of HCV RNA was not an independent predictor of sustained IFN efficacy in multivariate analysis. This finding could be based on the fact that HCV-non-1b correlated closely with lower levels of HCV, whereas HCV-1b was associated with higher levels of HCV (data not shown), and therefore multivariate analysis showed that pretreatment serum HCV RNA concentration was not an independent predictor.

Previous reports by Fukuda et al. [1997] have shown that a higher titer of HCV RNA and more severe hepatitis activity were significantly associated with less IFN receptor mRNA expression. However, our data show that neither serum HCV RNA concentration nor histologic grades affected the expression of IFN receptor mRNA. It has been reported that expression of the IFN-α receptor may be upregulated by treatment with IFN-γ in some cell lines, and a synergistic effect was found in response to human IFN-α [Isii and Tsukagoshi, 1989]. This finding suggests that the cytokine network may participate in the induction of IFN receptors and may explain the discrepancy between our and previous data on IFN receptor mRNA expression, although further study of these associations is necessary.

In the present study, serum ALT levels decreased significantly more from basal levels in patients positive for IFN receptor mRNA compared with negative patients, and a primary biochemical remission was obtained in all patients expressing IFN receptor mRNA. A recent study suggested that patients with high serum ALT levels were at high risk for hepatocellular carcinoma [Benvegnù and Alberti, 1996], and the cumulative incidence of hepatocellular carcinoma in patients showing transient response to IFN therapy was almost equal to that in sustained responders [Kasahara et al., 1998]. Therefore, we believe that IFN therapy may be a positive indicator for patients who express IFN receptor mRNA since medical interventions that limited disease activity may prevent or delay neoplastic transformation and tumor growth.

Recently, it was reported that, in patients with HCV-2a or HCV-2b infection, IFN receptor gene expression in the liver was associated significantly with the efficacy of IFN [Morita et al., 1998]. However, in a population of chronic hepatitis patients who had various HCV genotypes, our study demonstrated that IFN receptor mRNA expression in the liver and HCV-non-1b infection were closely associated with the efficacy of IFN therapy. It is concluded that the determination of IFN receptor expression in the liver before IFN treatment reliably predicts the short- and long-term outcome of chronic hepatitis caused by HCV and that appropriate therapeutic strategies may be designed according to hepatic IFN receptor mRNA expression.

REFERENCES

Aguet M. 1980. High-affinity binding of ^{125}I-labelled mouse interferon to a specific cell surface receptor. Nature 284:459–461.

Aguet M, Gresser I, Hovanessian AG, Bandu MT, Blanchard B, Blangy D. 1981. Specific high-affinity binding of ^{125}I-labeled mouse interferon to interferon resistant embryonal carcinoma cells in vitro. Virology 114:585–588.

Benvegnù L, Alberti A. 1996. Risk factors and prevention of hepatocellular carcinoma in HCV infection. Dig Dis Sci 41:49S–55S.

Chayama K, Tsubota A, Kobayashi M, Okamoto K, Hashimoto M, Miyano Y, Koike H, Kobayashi M, Koida I, Arase Y, Saitoh S, Suzuki Y, Murashima N, Ikeda K, Kumada H. 1997. Pretreatment virus load and multiple amino acid substitutions in the interferon sensitivity-determining region predict the outcome of interferon treatment in patients with chronic genotype 1b hepatitis C virus infection. Hepatology 25:745–749.

Colamonici OR, Pfeffer LM, D'alessandro F, Platanias LC, Gregory SA, Rosolen A, Nordan R, Cruciani RA, Diaz MO. 1992. Multichain structure of the IFN-α receptor on hematopoietic cells. J Immunol 148:2126–2132.

Davis GL. 1994. Prediction of response to interferon treatment of chronic hepatitis C. J Hepatol 21:1–3.

Davis GL, Balart LA, Schiff ER, Lindsay K, Bodenheimer HC Jr, Perrillo RP, Carey W, Jacobson IM, Payne J, Dienstag JL, Van Thiel DH, Tamburro C, Lefkowitch J, Albrecht J, Meschievitz C, Ortego TJ, Gibas A, Hepatitis Interventional Therapy Group. 1989. Treatment of chronic hepatitis C with recombinant interferon alpha: a multicenter randomized, controlled trial. N Engl J Med 321:1501–1506.

Di Bisceglie AM, Martin P, Kassianides C, Lisker-Melman M, Murray L, Waggoner J, Goodman Z, Banks SM, Hoofnagle JH. 1989. Recombinant interferon alpha therapy for chronic hepatitis C: a randomized, double-blind, placebo-controlled trial. N Engl J Med 321: 1506–1510.

Dienstag JL. 1983. Non-A, non-B hepatitis. I. Recognition, epidemiology, and clinical features. Gastroenterology 85:439–462.

Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. 1996. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 334:77–81.

Fukuda R, Ishimura N, Kushiyama Y, Moriyama N, Ishihara S, Nagasawa S, Miyake T, Niigaki M, Satoh S, Sakai S, Akagi S, Watanabe M, Fukumoto S. 1997. Effectiveness of interferon-alpha therapy in chronic hepatitis C is associated with the amount of interferon-alpha receptor mRNA in the liver. J Hepatol 26:455–461.

Groote JD, Desmet VJ, Gedigk P, Korb G, Popper H, Poulsen H, Scheuer PJ, Schmid M, Thaler H, Uehlinger E. 1968. A classification of chronic hepatitis. Lancet 2:626–628.

Hagiwara H, Hayashi N, Mita E, Takehara T, Kasahara A, Fusamoto H, Kamada T. 1993. Quantitative analysis of hepatitis C virus RNA in serum during interferon alpha therapy. Gastroenterology 104:877–883.

Hoofnagle JH, Mullen KD, Jones DB, Rustgi V, Bisceglie AD, Peters M, Waggoner JG, Park Y, Jones EA. 1986. Treatment of chronic non-A, non-B hepatitis with recombinant human alpha interferon: a preliminary report. N Engl J Med 315:1575–1578.

Isii Y, Tsukagoshi S. 1989. The synergistic effect of human recombinant interferon-α_{2a} in combination with interferon-γ and the induction of interferon-α_{2a} receptor by interferon-γ. J Pharmacobiodyn 12:299–304.

Kasahara A, Hayashi N, Mochizuki K, Takayanagi M, Yoshioka K, Kakumu S, Iijima A, Urushihara A, Kiyosawa K, Okuda M, Hino K, Okita K, Osaka Liver Disease Study Group. 1998. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Hepatology 27: 1394–1402.

Kato N, Hijikata M, Ootsuyama Y, Nakagawa M, Ohkoshi S, Sugimura T, Shimotohno K. 1990. Molecular cloning of the human hepatitis C virus genome from Japanese patients with non-A, non-B hepatitis. Proc Natl Acad Sci USA 87:9524–9528.

Kiyosawa K, Sodeyama T, Tanaka E, Gibo Y, Yoshizawa K, Nakano Y, Furuta S, Akahane Y, Nishioka K, Purcell RH, Alter HJ. 1990. Interrelationship of blood transfusion, non-A, non-B hepatitis and hepatocellular carcinoma: analysis by detection of antibody to hepatitis C virus. Hepatology 12:671–675.

Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. 1981. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1:431–435.

Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M. 1989. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. Science 244:362–364.

Lutfalla G, Holland SJ, Cinato E, Monneron D, Reboul J, Rogers NC, Smith JM, Stark GR, Gardiner K, Mogensen KE, Kerr IM, Uzé G. 1995. Mutant U5A cells are complemented by an interferon-α β receptor subunit generated by alternative processing of a new member of a cytokine receptor gene cluster. EMBO J 14:5100–5108.

Marcellin P, Boyer N, Giostra E, Degott C, Courouce AM, Degos F, Coppere H, Cales P, Couzigou P, Benhamou JP. 1991. Recombinant human α-interferon in patients with chronic non-A, non-B hepatitis: a multicenter randomized controlled trial from France. Hepatology 13:393–397.

Morita K, Tanaka K, Saito S, Kitamura T, Kondo M, Sakaguchi T, Morimoto M, Sekihara H. 1998. Expression of interferon receptor genes (IFNAR1 and IFNAR2 mRNA) in the liver may predict outcome after interferon therapy in patients with chronic genotype 2a or 2b hepatitis C virus infection. J Clin Gastroenterol 26:135–140.

Novick D, Cohen B, Rubinstein M. 1994. The human interferon α/β receptor: characterization and molecular cloning. Cell 77:391–400.

Squadrito G, Leone F, Sartori M, Nalpas B, Berthelot P, Raimondo G, Pol S, Bréchot C. 1997. Mutations in the nonstructural 5A region of hepatitis C virus and response of chronic hepatitis to interferon alpha. Gastroenterology 113:567–572.

Suzuki T, Tanaka E, Matsumoto A, Urushihara A, Sodeyama T. 1995. Usefulness of simple assays for serum concentration of hepatitis C virus RNA and HCV genotype in predicting the response of patients with chronic hepatitis C to interferon α2a therapy. J Med Virol 46:162–168.

Tine' F, Magrin S, Crazi' A, Pagliaro L. 1991. Interferon for non-A, non-B chronic hepatitis: a meta-analysis of randomised clinical trials. J Hepatol 13:192–199.

Uzé G, Lutfalla G, Gresser I. 1990. Genetic transfer of a functional human interferon α receptor into mouse cells: cloning and expression of its cDNA. Cell 60:225–234.

Yatsuhashi H, Yamasaki K, Aritomi T, Carmen PM, Inoue O, Koga M, Yano M. 1997. Quantitative analysis of interferon α/β receptor mRNA in the liver of patients with chronic hepatitis C: correlation with serum hepatitis C virus-RNA levels and response to treatment with interferon. J Gastroenterol Hepatol 12:460–467.

Zeuzem S, Lee J-H, Roth WK. 1997. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alpha. Hepatology 25:740–744.